Report of the Haffkine Institute

For the Year 1928

LIBRARY OF THE BOMBAY BRANCH OF THE ROYAL ASIATIC SOCIETY.

BY

MAJOR S. S. SOKHEY, I.M.S.,
Offg. Director, Haffkine Institute

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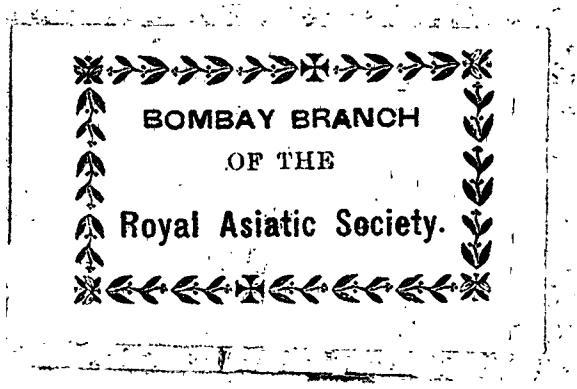

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REPORT OF THE HAFFKINE INSTITUTE, 1928.

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Insert the following note below Table VIII on page 43 :—

Further statistics if required will be supplied on application to the Director, Haffkine Institute, Parel, Bombay.

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LIBRARY OF THE BOMBAY BRANCH OF THE ROYAL ASIATIC SOCIETY

REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1928.

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Lt.-Col. F. P. Mackie, K.H.S., O.B.E., M.D., M.Sc., D.P.H., F.R.C.S., F.R.C.P., I.M.S., from 1st January to 23rd March 1928.

Assistant Directors.

Major S. S. Sokhey, M.A., B.Sc., M.D. (Edin.), D.T.M. & H. (Eng.), I.M.S., in charge Biochemical Unit; acted as Director from 24th March 1928 to 31st December 1928.

Major W. J. Webster, M.C., M.B., D.T.M. & H. (Eng.), I.M.S., Officiating Assistant Director from 1st July to 31st December 1928.

Mr. S. N. Goré, L.M. & S.

Pharmacological Unit.

Rev. Father J. F. Caius, S.J., F.L.S., M.S.C.I. (Paris).

Dr. K. S. Mhaskar, M.A., B.Sc., M.D, D.P.H., D.T.M. & H.

Biochemical Unit.

Mr. M. A. Malandkar, M.A., M.Sc.

Mr. S. K. Gokhale, B.A. (Hons.), M.Sc., A.I.I.Sc.

Anti-rabic Department.

Military Assistant Surgeon H. M. La Frenais, I.M.D.

Mr. R. K. Gokhale, S.M.S.

Anti-plague Inquiry.

Dr. B. P. B. Naidu, M.D. (Edin.), M.Hy., D.P.H., D.T.M. (L'pool).

Dr. J. H. Wadia, B.A. (Hons.), B.Sc. (Bom.), Ph.D. (Cantab.)

Mr. H. S. Billimoria, M.B., B.S., D.T.M. (L'pool).

Jamadar Shamsher Jung, I.M.D.

Jamadar F. Jasudasan, I.M.D.

Inquiry on Maternal Morbidity and Mortality in Childbirth.

Dr. Margaret Balfour, C.B.E., M.D.

Dr. M. A. B. Milne, W.M.S., 1st January to 30th September 1928.

Dr. L. Wills, M.D., from 1st October 1928 to 31st December 1928.

Mrs. Emanuelov.

Dr. M. Mehta, Ph.D., D.Sc. (Lond.)

Mrs. G. Talpade, M.B., B.S.

Tuberculosis Inquiry.

Dr. M. B. Soparkar, B.Hy., M.D. (Bom.), from 24th August 1928 to 31st December 1928.

Jamadar C. S. Dhilon, I.M.D., from 24th August 1928 to 31st December 1928.

Medical and Scientific Assistants.

Khan Bahadur C. R. Avari, B.M.S. (General Diagnostic Department).
Mr. G. D. Chitre, L.M. & S., B.M.S. (Plague Research).
Mr. N. P. Dalal, M.B., B.S., D.P.H., D.T.M. & H. (Eng.).
Mr. T. G. Akula, S.M.S. (Morbid Histology and Malaria).
Mr. K. H. Bharucha, B.A., B.Sc. (Public Health Chemistry).

Chief Technical Assistants.

Mr. A. Petty (Plague Vaccine).
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„ J. Mallett (do.).
„ F. R. Mistri—Head Clerk.
„ G. G. Subnis—Accountant.
„ R. D. Panwaikar, A.M.—Artist and Photographer.
„ S. V. Nadkarni—Storekeeper.
„ H. R. Rajadhyax—Packing.
„ H. J. Parelkar—Engineer.
Shaik Mahomedalli—Senior Media Maker.
Junior staff, subordinates and menials number 120.

General Review.

Plague Vaccine.—During the current year about two million doses of Haffkine's Plague Prophylactic were sent out; this is a considerable increase on the last year's demand. The United Provinces of Agra and Oudh and the Punjab are still the largest consumers.

The process of manufacture of the prophylactic has remained unaltered. But the discovery by Dr. Goré of the fact that the blood agar is the medium of choice for the growth of *B. pestis* has enabled us to detect with greater certainty contaminating organisms in the prepared vaccine, and thus secure greater purity of the finished product. Dr. Gore has found that the growth of *B. pestis* is more profuse on blood agar than on nutrient agar. If dilutions of broth culture of *B. pestis* are made, a point is reached when the dilution planted on nutrient agar shows little or no growth, while blood agar shows numerous colonies. This dilution in the case of six weeks' cultures of *B. pestis* in broth—Haffkine's Prophylactic—is roughly 1 in 1,000. A small quantity of culture to be tested is diluted as indicated above and is planted on blood agar and nutrient agar. If after 48 hours incubation the agar slope shows any growth at all, contamination is suspected and further exhaustive tests are made.

This test is now being used as a routine measure for testing the purity of the seed cultures and the fully incubated vaccine. Until this test was introduced, besides the sugar reaction, the purity was judged by the appearance of agar slopes planted with undiluted cultures. The slopes were incubated for 48 to 72 hours, and if they

presented a ground glass semi-translucent appearance they were taken to indicate pure plague cultures; moreover in the sugar test *B. pestis* was expected to ferment without the production of gas, glucose and mannite only. We now know the ground glass appearance of the agar slopes does not necessarily indicate pure *B. pestis* growth. Some contaminants, especially a Gram positive coccus, escape detection, because they in no way disturb the so-called ground glass appearance of the agar slopes. Those common contaminants produce no change in the sugars, and so again escape detection. All these organisms are easily detected by the present blood agar test. If any growth appears on agar slopes it raises a suspicion of contamination, and the examination of discrete colonies which are regularly obtained on blood agar decides the point.

Making use of Gore's blood agar slopes, Major Sokhey and Dr. La Frenais made a number of quantitative determinations bearing on the manufacture of Haffkine's prophylactic. They have found that a wide range of pH of the medium gives good growths of *B. pestis*—pH 6·2 to 7·4, with a slight advantage in favour of 7·0. In the broth ordinarily used for the preparation of the prophylactic the growth of *B. pestis* comes to an end at the termination of three weeks. The addition of ½ cc. of rabbit blood per 10 cc. of broth makes the growth much more profuse than that in broth without blood. It was also found that although the growth is profuse, it loses its virulence rapidly. After about 40 subcultures in blood broth, each of 48 days duration, 40 million organisms failed to kill rats, where the 50 organisms of the original growth in nutrient broth killed cent per cent of the rats.

Major Sokhey and Dr. La Frenais also investigated into the validity of the routine test in use for testing the immunising powers of the various brews of the prophylactic manufactured. The results of duplicate tests on the same brews varied sometimes by 300 per cent and more, and showed that the test had no value as a measure of the immunising properties of the prophylactic. They found the test dose of 0·003 mg. of plague-spleen used in the routine test was a very variable quantity as far as the number of plague bacilli contained in it went. An actual count of seven test viruses made and used by Dr. Naidu in his work showed that the number of organisms in 0·003 mg. of his test virus spleen varied from 120 to 46,000. In place of the routine technique 23 experiments were performed with 11 brews, using a test virus containing organisms between 490 and 1,000. The results of duplicates were much closer together, but still the variation was great enough to render the test valueless. Work on the question is still proceeding. It is proposed to place this work before a committee of experts to recommend some minor changes in the manufacture of the plague prophylactic.

Parasitology of Plague.—The Plague Expert Committee of the Health Organisation of the League of Nations at their first session in Calcutta in December 1927 laid down the lines of future research in plague and they laid stress on the "Investigation into the

comparative epidemiological role of the various species of fleas in plague transmission in selected areas of India." This investigation was taken up by Major Webster and Dr. Chitre in August, and the work is still proceeding. But it may be mentioned that up to now they have secured successful transmission experiments with all the three species of fleas found in Bombay, *i.e.*, *Xenopsylla cheopis*, *X. astia*, and *X. brasiliensis*. The successful transmission with *X. brasiliensis* is interesting as this species has not previously been proved to be a vector of plague in India.

Anti-plague Serum.—The work by Dr. Naidu and his assistants reported last year has been continued during the year. Following on the successful experiments in the production of a potent anti-plague serum in rabbits; calves, and sheep have been selected as the experimental animals to obtain larger quantities of the serum. The work is proceeding, and the results obtained so far are promising.

The Pharmacological Department.—Work on the chemotherapy of plague has been continued. Two classes of drugs are claiming the attention of the department:—(1) the three dihydroxybenzenes—catechol, resorcinol, and quinol, and (2) mercurated phenols and mecurated amines. Forty-eight mercurial compounds have been synthesized and the department is now directing its attention to animal experiments. The work is not sufficiently advanced yet to record any results.

The indigenous drugs section has been studying *Garcinia mangostana, Terminalia sp.*, and *Gymnema sylvestre*. The results obtained are to be found in the body of the report. These investigations are of scientific value; but those who expect the immediate discovery of wonderful therapeutic agents in large numbers to replace the remedies in use at present are sure to be disappointed. Investigations into the nature of indigenous drugs is a problem which involves much time and labour. It is a matter of regret that the pharmacological department is understaffed.

The Biochemical Department.—Researches in Anæmia by Major Sokhey and his assistants have revealed the great prevalence of anæmia resembling pernicious anæmia among men and women in Bombay. Their studies show that anæmia of sprue and of pregnancy have great resemblance to pernicious anæmia, and they believe that the so-called anæmia of pregnancy is not a distinct entity, but merely an aggravated condition of anæmia commonly present in non-pregnant women.

The work on basal metabolic rate was continued during the year and results tend to show that the average rate for India is about 10 per cent. lower than that for Europeans. This they are inclined to believe is due to climatic factors in the widest sense of the term.

The department is also establishing normal standards for various substances usually investigated in diagnostic medicine in the chemical analysis of blood and urine. The department was also engaged on some problems connected with the manufacture of plague vaccine and noted above. Besides the research work th

department has proved of value in aiding the more advanced physicians in their diagnostic work. It is now practically impossible to practice modern medicine without the aid of biochemical analytical work.

The Antirabic Department.—The statistical figures for the department given in the body of the report show that the value of the antirabic centre is well understood by the public, and that full use is made of the centre. Against 718 persons who received treatment, 458 persons reported here who did not need any anti rabic treatment. They were advised regarding keeping the offending animal under observation, and their wounds were dressed.

Thorough disinfection of the wounds was carried out as a routine measure in the treatment of all cases who reported for treatment within a reasonable time after injury. The death rate among patients so treated was 0·43 per cent. as compared with the death rate of 1·63 per cent. among patients not so treated for various reasons.

It is to be regretted that the majority of the outcentres which receive antirabic vaccine from this Institute have not furnished statistical returns for the treatments carried out by them. They should realize that antirabic treatment has not reached finality and that it is still in an experimental stage. Unless accurate records are kept of all the cases treated, and statistical returns supplied, efforts at the improvements in the treatment are hampered.

Researches on Sprue.—A comprehensive study of this disease started by Col. Mackie and his colleagues in 1923 and resumed in 1925, was brought to a close this year when Col. Mackie left the Institute to take up the appointment of Public Health Commissioner with the Government of India. The broad conclusions arrived at by these researches were summarised in the last report and most of the papers foreshadowed have either appeared during the year or are in press. A list of these papers is given under the heading "List of papers published during the year."

Enquiry on Maternal Mortality in Childbirth.—The investigation was continued throughout the year. Dr. Balfour was on leave from March to October, and her place was taken by Dr. Milne. In October the staff of this enquiry was strengthened by the employment of Dr. Lucy Wills. Dr. Milne and Dr. Mehta continued the routine examination of cases of anæmia of pregnancy and also carried out a series of animal experiments. *B. welchii* has been considered by some to be the possible etiological factor, but the animal experiments done here give no evidence to that effect. Nor do animal experiments with streptococcus give evidence that this organism is of any etiological significance.

Dr. Wills has studied 50 cases of anæmia of pregnancy, and four non-pregnant cases with similar blood picture. She finds the blood counts and pictures of the two categories to be similar. Free hydrochloric acid was present in almost all cases, and they all responded to liver treatment equally well. Dr. Wills has reason to

believe that the so-called anæmia of pregnancy is not a pathological entity. The macrocytic anæmia, evidently quite common among the women in Bombay, is made worse by the strain of pregnancy. Dr. Wills is also inclined to the view that this type of anæmia very likely is a deficiency disease, and she is taking up animal experiments to test this hypothesis.

Tuberculosis Enquiry.—At the end of August this enquiry, under the charge of Dr. Soparkar, was transferred from Kasauli to Bombay. Dr. Soparkar and his assistants have been engaged in an investigation of the nature of organisms causing the surgical type of tuberculosis in human beings. This type of disease is known to be caused by bovine bacillus, but in India they have come across only one case out of a series of more than 65, which was due to the bovine bacillus.

Their preliminary experiments with B. C. G. have not borne out the claims made for its immunising power. They are also carrying on experiments upon transformation of tubercle bacilli of one type into another, but they have arrived at no conclusions yet.

The Indian Research Fund Association.—The cost of the plague, anæmia, maternal mortality and tuberculosis enquiries amounting to Rs. 1,37,677 was borne by the Research Fund Association. In future years the situation as regards grants from the Association is likely to be altered radically. The Fletcher Committee which was referred to in the last year's report, has recommended the establishment of the Central Research Institute at Dehra Dun, and the Rockefeller Foundation has decided to build the Public Health Institute at Calcutta.

So the position is that Bombay is to receive no aid from any outside agency to help it to make provision for post graduate training in Tropical Medicine or Public Health and Hygiene. Bombay is as important a centre of medical education as any in India, but its promising graduates must continue to go elsewhere to complete their training in Tropical Medicine. Calcutta has a flourishing School of Tropical Medicine and will soon have a first class Institute of Public Health and Hygiene; while Bombay has neither. One School of Tropical Medicine is not enough for the whole of India, and it is high time for Bombay to make provision for itself.

It is a melancholy fact that Bombay is making no headway in providing for research work in Medicine. So far all the research carried on at this Institute has been subsidised by the Research Fund Association, and had it not been for substantial help from that Association the Pharmacological and Biochemical Units of this Institute would possibly not yet be in existence. Should the Research Fund Association stop their contributions, most of the research work now in progress should have to be abandoned, with little hope of being resumed in the near future.

The Social Life of the Institute.—The Critics Club:—This club came into existence in June 1924 at the instance of the Director and has for its members the medical and scientific staff of the Institute. Its objects are (1) to increase the social relations between the staff of the different departments, (2) to hold meetings when the researches carried on in different departments may be reviewed and criticised and (3) to act as stimulus for the junior members of the staff to undertake research work. The following subjects were discussed during the year:—

(1) The utility of determining the production of Hydrogen Sulphide and Indole in the routine bacteriological examination of water by means of the cotton wool plug technique, by Gore.

(2) The value of early and thorough cleansing of wounds before antirabic treatment is commenced, by La Frenais.

(3) Researches to be carried out at the Institute for the ensuing year, by Mackie.

(4) Reorganisation of the Institute Library, by Sokhey.

(5) The comparative estimation of the quantity of growth of *B. pestis* on blood agar and acid agar slopes, by Gore.

(6) Chemo-therapy of Mercuro-Phenolic Drugs, by Caius, Bharucha and Wadia; and the Pharmacological Properties of *Gymnema sylvestre*, by Mhaskar.

(7) Modern methods of investigating Malaria, by Covell.

(8) Clinical signs and symptoms of cases of anæmia of pregnancy together with bacteriological and *post mortem* findings, by Milne and Mehta.

(9) Some of the factors involved in the production of a potent antiplague serum, by Naidu.

The Critics Club maintains a tennis court, which not only provides an inexpensive recreation for the members and their families, but promotes social intercourse between them. This tennis club continues to grow in popularity and the annual tennis tournament was again a huge success.

The Cricket Club.—This club was started primarily for the benefit of the personnel of the Institute who number about 150. During the two years of its existence it has firmly established itself in their esteem, and has been very valuable in fostering among them an *esprit de corps*. During the last season, the club played 18 matches, won 2, lost 9, and the remainder ended in a draw.

The atmosphere of co-operation and cordiality, so essential for scientific endeavour, was maintained without blemish by the staff. The acting director received unstinted and loyal support of his colleagues which made his work easy and profitable.

HAFFKINE'S PLAGUE PROPHYLACTIC VACCINE.

1. *Manufacture.*—Since the discovery of the prophylactic in Bombay in 1897 and its introduction as a measure of personal protection, the attitude of the public has undergone a profound change for the better; its efficacy as a protective measure is now universally recognised, and anti-plague inoculation is a popular measure in almost all districts. The demand for the vaccine, therefore, has steadily been on the increase.

From the opening of the laboratory to the end of 1928, 31,130,455 doses have been sent out. The output of the vaccine has been as follows :—

Year	Doses	Year	Doses
1897-1901	2,867,041	1917-1921	6,667,894
1902-1906	3,455,537	1922-1926	7,202,636
1907-1911	3,584,262	1927	825,310
1912-1916	4,567,366		

During the year 1928, the output was 1,960,409 doses; and the number of doses of antiplague vaccine dispatched monthly during the past two years are detailed :—

Month	*1927*	*1928*
January	75,012	164,072
February	88,350	365,196
March	65,050	384,311
April	49,878	202,526
May	2,676	60,264
June	8,556	16,464
July	18,720	36,518
August	46,596	56,598
September	119,136	196,356
October	87,808	130,200
November	133,850	119,796
December	129,678	228,108
Total number of doses	825,310	1,960,409

2. *Distribution.*—The geographical distribution of the vaccine in India and to places outside India during the years 1927 and 1928 is shown below :—

	1927	*1928*
British India—		
Bombay Presidency	73,330	255,787
Madras Presidency	117,216	130,416
Bengal, Behar and Assam	30,708	36,738
United Provinces of Agra and Oudh	102,660	521,596
The Punjab	8,148	466,494
North-West Frontier Provinces	3,012	2,686
Central Provinces and Berar	92,610	106,110
Delhi Province	702	1,986
Burma	115,332	80,514

	1927	1928		
Indian States—				
Kathiawar	9,384	6,108		
Southern Maratha Country ...	2,376	18,048		
Savantwadi	1,116	1,770		
Baroda	870	2,256		
Palanpur	204	...		
Cutch	1,488	7,860		
Hyderabad	103,226	120,750		
Mysore	85,842	136,662		
Travancore	30,752	18,402		
Benares	252	2,400		
Patiala	8,016	20,214		
Jhind	2,070	3,012		
Faridkot	504	1,008		
Jammu	10,200	2,658		
Bhopal	300	...		
Jaipur	504	462		
Bikaner	6,528	402		
Jodhpur	5,124	...		
Indore	.....	102		
Rewa	.....	2,016		
Dhar	.....	12		
Dewas Junior	.....	12		
Darbhanga	.....	504		
Rampur State	.....	3,204		
Alwar State	.....	408		
Maihar State	.....	750		
Sandur State	.....	234		
Ramandrug	.....	810		
Tonk, Rajputana	.....	54		
Nabha State	.....	204		
Rutlam	.....	72		
			268,756	350,394
	268,756	350,394		

	1927	1928
Portuguese Territory in India	660	120
Countries outside India—		
Abyssinia—Addis Ababa	.....	2,004
Arabia—Kamaran	1,008	.....
British Somali Land—Berbera	.....	2,010
Ceylon—Colombo	72	.....
China—		
Sienya Fukien	.....	504
Hinghwa Foochow	504	2,202
Shanghai	2,004	.....
East Africa—Nairobi	54	.....
Federated Malay States—		
Kualalampur	5,010	.....
Singapore	.....	846
Iraq—		
Baghdad	524	.....
Busrah	3,000	.....
Total number of doses ...	825,310	1,960,409

Examination of Rats for Plague.

1

Rats received at the Institute from the Municipality during 1928

Month	Alive	Dead	Total
January	18,504	35,773	54,277
February	17,277	33,786	51,063
March	19,196	35,746	54,942
April	18,867	33,214	52,081
May	17,727	30,979	48,706
June	15,059	30,559	45,618
July	16,501	30,838	47,339
August	18,699	29,003	47,702
September	19,999	29,526	49,525
October	25,663	36,137	61,800
November	22,740	32,278	55,018
December	20,717	35,208	55,925
Total ...	230,949	393,047	623,996

2

Examination of dead rats for plague during 1928

Month	Dead rats examined	Plague infected rats	Rattus norvegicus	Rattus rattus
January	25,300	220	189	31
February	26,256	313	258	55
March	23,005	451	380	71
April	22,501	511	426	85
May	21,737	340	301	39
June	20,739	124	121	3
July	22,565	63	60	3
August	21,565	39	35	4
September	19,183	48	43	5
October	25,878	55	47	8
November	23,354	33	32	1
December	24,012	25	25	...
Total ...	276,095	2,222	1,917	305

Relation of human plague deaths to plague deaths among rats

Month	Human plague deaths	Rats examined *post mortem*	Plague infected rats	Percentage mortality
January	6	25,300	220	0·9
February	22	26,256	313	1·2
March	27	23,005	451	2·0
April	61	22,501	511	2·3
May	37	21,737	340	1·6
June	9	20,739	124	0·6
July	2	22,565	63	0·3
August	3	21,565	39	0·2
September	6	19,183	48	0·3
October	5	25,878	55	0·2
November	5	23,354	33	0·1
December	1	24,012	25	0·1
Total ...	184	276,095	2,222	0·80

Pharmacological Laboratory.

I. Indigenous Drugs.

1. *Holarrhena antidysenterica.*—In the Indian Medical Gazette for August 1928, p.479, Knowles and others state that "the simple administration of Kurchi orally gave surprisingly good results....." Barring the word 'surprisingly' we, of Bombay, had already said the same in May 1927.

2. *Garcinia mangostana.*—A native of the Straits, this tree has been successfully cultivated at a few places in the Madras Presidency. The dried rind of the entire fruit is largely used as a remedy for diarrhoea and dysentery. Our clinical results show that mangosteen has antidiarrhoeal properties less pronounced than those of *Holarrhena antidysenterica.*

3. *Terminalia sp.*—A comparative study of the barks of the Indian species of genus *Terminalia* as cardiac tonics has brought out the fact that *T. arjuna* has no marked stimulant effect on the heart.

4. *Gymnema sylvestre.*—The study of this plant based on the report that diabetic persons in Bombay were using the leaves with some benefit, was taken up in October 1924.

The chemical examination of the leaves has proved a matter of more than ordinary difficulty and, although much has been done, we are still far from a definite knowledge of their composition. The following points may, however, be recorded in this report :—(1) ash amounting to 11·45 per cent. with 0·77 phosphorus (P_2O_5), 0·62 iron (Fe_2O_3), 0·15 manganese (Mn) ; (2) the presence of hentriacontane, pentatriacontane, tartaric acid, and inosite ; (3) the presence of anthraquinone derivatives allied to those of aloe and rhubarb ; (4) the absence of any insulin like substance ; (5) the absence of any substance which destroys glucose *in vitro.*

The pharmacodynamics of the drug have been determined from the action of the leaves themselves, extracts, and fractions of the extracts. The salient points are :—(1) variations brought about in the blood sugar content of rabbits after oral administration of the leaves and a decoction of the leaves (as much as 16 per cent.), or hypodermic injection of extracts (to 34 per cent. lowering) ; (2) the lowering is not proportional to the amount injected, and the animals do not die of hypoglycoemia ; (3) the antisaccharine fractions—those which destroy the taste sensation for sugar—raise the sugar content of the blood from 10 to 30 per cent. (4) the non-antisaccharine fractions stimulate bile secretion and pancreatic secretion, and increase oxygen consumption in animals under deep anesthesia ; (5) increase of blood pressure by stimulation of the heart muscle and peripheral vaso-contriction ; (6) stimulation of intestinal and uterine muscle fibres ; (7) purgative properties.

II. Chemotherapy of Plague.

The drugs to be tried on plague-infected rabbits fall within two classes :—(1) the three dihydroxy benzenes—catechol, resorcinol, and quinol ; (2) mercurated phenols and mercurated amines.

48 mercurial compounds have been synthesized ; 13 of them have been purified, and the toxicity of 8 of these has been determined. The difficulties encountered in the preparation and purification of the drugs and the determination of their toxicity were of the type usually associated with this kind of work and could not, therefore, be called a disturbing factor.

Not so, however, the difficulties which had to be faced when determining the germicidal value of the drugs, and testing their efficiency on plague-infected animals. Uncertainty as to the number of organisms sowed or infected, and uncertainty as to their virulence left the enquiry without a solid scientific basis. These obstacles have been partly surmounted by realising partly the possibility of infecting or sowing a definite number of bacilli of known virulence. Thus we have been able to determine the time at which septicaemia regularly sets in and the time which elapses till death occurs. If we take the time intervening between the onset of septicaemia and death to represent individual resistance, we find that this resistance is fairly constant for a given breed, but seems to differ widely for different breeds. Experiments are still

in progress to settle this last point which is of fundamental importance for the study of the chemotherapeutical treatment of plague.

III. Earth-eating and Salt-licking.

Earth-eating is a habit which overspreads all countries and breaks ethnological boundaries of every sort; it is found among the people of every continent, and apparently of almost every race. In India it extends throughout the length and breadth of the land, and is indulged in by both the sexes, at any time of life, in all states and grades of society. There are as many as six theories to account for this practice which is known by the name of "geophagy", but none of them is satisfactory.

We believe that this widespread craving for earth of some kind is the psychological expression of a physiological need, that the habit primarily rests on a physiological basis. We know that wild beasts collect in numbers to lick or eat earth from definite areas called "licks". We are inclined to attribute earth-eating among men and salt-licking among wild animals primarily to a deficiency in one or more minerals. A superficial examination of the soils sought after by wild animals point to the fact that they are closely related to, if not the same as, the materials used by earth-eaters. One thing which stands out prominently is that, whatever it is the wild animals seek in the salt-licks, it is not sodium chloride. What it is may be revealed by a careful analysis of numerous samples of soils from the tracts visited by them. This is what we are now doing.

IV. Scorpion Venom.

If stung by a scorpion, what chance has a man got? This is one of the questions we have often been asked during three decades of almost daily contact with professional men of high and low degree, and with the various zones of culture making up our citizen population. Our answer has invariably been the same, and our sanity has invariably been doubted.

The purport of our enquiry is primarily to determine the toxic properties of the venom of Indian scorpions, and incidentally to vindicate the soundness of our mental health. What is mainly required to carry the work to a successful issue is sufficiency of material. Thanks to the Bombay Natural History Society we are receiving live specimens from all parts of India and we are daily adding to our material. When dried, bottled, and preserved in the dark, scorpion venom keeps indefinitely.

Biochemical Unit.

I. *Anæmia Enquiry.*—During the study of sprue it was noticed that anæmia was a very prominent feature of this disease, and that in some cases at least, it had close resemblance to the blood condition in pernicious Anæmia. Our colleagues in charge of the Maternal Mortality Enquiry had also observed that a severe anæmia with normal or high colour index was one of the very important

causes of death during pregnancy in Bombay. We, therefore, took up an intensive study of macrocytic anæmias with high colour index, with a view to ascertain in what respects sprue and severe anæmias of pregnancy differed from pernicious anæmia, and how the three conditions responded to the liver treatment. Our plan of work has been to take up all cases of macrocytic anæmias with complete corpuscular complement of hæmoglobin that are brought to our notice. Each case is investigated and if a cause, like malaria, chronic sepsis, etc., for the anæmia is discovered the case is eliminated from further study. The others are classified as pernicious anæmia, sprue, severe anæmia of pregnancy, and cases resembling pernicious anæmia, but not definitely falling in any of the other three categories. After the preliminary examinations the cases are put on "liver treatment" and examined every week until the blood condition becomes normal. The following examinations are made every week:—cell counts, hæmoglobin determination, cell volume determination; colour, volume and saturation indices are calculated; blood pictures from smears are recorded, and differential cell counts made; blood is examined chemically to determine non-protein nitrogen, urea nitrogen, creatinine, cholesterol, fibrin, albumin, and globulin; and the Van den Bergh reaction is tested. Gastric contents are examined periodically, and notes made of any nervous symptoms or signs shown by the patients.

Macrocytic anæmias must be very common in Bombay, for out of 57 cases of severe anæmia we took up, 50 were of the macrocytic variety, and all these cases were in the wards of two physicians alone, Dr. J. D. Padshah and Col. Hamilton, to whom we are indebted for the facilities and the assistance they have given us in this enquiry. However, we were able to carry our studies to completion in only 16 cases, and to partial completion in 6. It takes 8 to 10 weeks for cases under liver treatment to come up to a cell count of between 4 and 5 millions, and most of the hospital patients are poor wage earners and cannot be induced to stay in hospital after they have started "getting better". These 22 cases we have provisionally classified as follows:—

Pernicious anæmia	11
Sprue	4
Severe anæmia of pregnancy	7

Our investigation is still proceeding, but some salient points may be mentioned at this stage.

(*a*) *Blood.*—Severe oligocythemia was a marked feature and cell counts as low as half a million cells were encountered in all the three categories. Anisocytosis, with marked macrocytosis, and cell volume index more than 1 was a feature common to all. Red cells had a full complement of hæmoglobin and colour, and saturation indices worked out at 1 or over 1. Smear pictures were mostly indistinguishable in the three conditions. In one case of sprue such was not the case and the picture resembled that of so called

"aplastic anæmia". But one week's "liver treatment" raised the Van den Bergh reaction, increased the cell count, and also produced a smear picture of classical pernicious anæmia. The globulin content of plasma is low in all these three conditions.

(*b*) *Digestive System.*—Glossitis was either present at the time of examination or a history could be obtained in all our cases of pernicious anæmia and sprue and some cases of anæmia of pregnancy.

Free hydrochloric acid was absent from the gastric contents of all our cases of pernicious anæmia. Hypochlorhydria was noted in some cases of pregnancy anæmia. In two of our cases of pernicious anæmia free hydrochloric acid reappeared in the gastric contents when the cell count had become normal. It is to be noted that both these patients were young—16 and 21 years old.

Diarrhoea was present in most cases of pernicious anæmia and anæmias of pregnancy, and cases were classified as sprue only if they either had or gave a history of having had morning characteristic diarrhoea.

(*c*) *Nervous system.*—Subjective or objective nervous symptoms were noticed in only 3 cases of pernicious anæmia. But we wish to point out that histories of subjective symptoms given by the class of patients we are dealing with are very unreliable.

(*d*) *Response to liver treatment.*—However unknown and varied the factors causing these conditions may be, one thing seems certain that all cases respond to liver treatment. Cases that were moribund with a cell count as low as half a million exhibited a dramatic change in as short a period as five days. In all cases the cell counts reached 4 or 5 millions in about 9 weeks; diarrhoea, anurexia, fever, disappeared in a week or so. Anæmias of pregnant women disappeared and normal labours resulted.

II. *Basal Metabolism.*—During the period under review 87 determinations of basal metabolism were done. Basal Metabolic Rate for this series of Indians works out at 11·4 per cent. lower than the Du Bois Standards for Europeans, and 7·8 per cent. lower than the Haris-Benedict Standards. All these determinations were done by the open circuit method using a modified Tissot gasometer. We propose to complete our series of 100 subjects and also to repeat some of the determinations on a closed circuit apparatus before publishing our results.

Our work so far indicates that the Basal Metabolic Rate for Indians is lower than that found for Westerners residing in Europe and America. The lower rate may possibly be due to the European surface area and height and weight factors not being applicable to Indians. Work done in China does not show that such is the case. Further, the few determinations that we have done on Europeans residing in Bombay have also given lower results. We are, therefore, inclined to the view that climate in the widest sense of the term, *i.e.*, prevailing temperature and humidity, and its effect on the

type and amount of food consumed, and the exercise taken, is a factor. In our own cases we have observed that the nitrogen excretion in urine is on an average about 6 gm. per day indicating low protein consumption.

III. *Standards for normals.*—In taking up any chemical analysis for diagnostic work in medicine, one is always brought up against the question—are the Western figures for normals applicable to Indians. There is only one way to answer the question and that is to examine a large number of Indians enjoying good health. We have therefore started examining Indians for establishing standards. We have already performed a large number of blood analyses for non-protein nitrogen, urea, uric acid, creatinine, phosphates, cholesterols, fatty acids, plasma proteins—fibrin, globulin and albumin. Cell counts and hæmoglobin standards for blood are also being worked up.

Chemical Department.

Waters.—During the year under report 78 samples of water were examined chemically, of which 27 were examined for their mineral constituents as well. The sources from which they were obtained, and the extremes of variations in total solids and chlorine, are shown in the table. The samples are grouped into four divisions, *viz.*, surface waters, bores, wells, and rivers; the first including lakes, tanks, reservoirs, jack-wells and tap supplies.

Source	No.	Total solids		Chlorine		Potability	
		Highest	Lowest	Highest	Lowest	Positive	Negative
Surface waters ...	37	54·0	6·4	6·8	Nil.	11	26
Bores ...	19	2000·0	86·0	1200·0	32·0	3	16
Wells ...	18	276·0	31·6	80·0	3·2	1	17
Rivers ...	4	46·8	7·2	5·2	1·0	Nil.	4

Of the 78 samples examined only 15 were found to be chemically fit for potable purposes, when judged by the standards laid down in English text-books on Hygiene.

Other Work.—Three samples of effluent, four samples of milk (including one sample of human milk), and one sample of ghee were examined chemically. The department supplied stains and standard solutions to some departments of the institute and various laboratories in the Presidency as usual.

Bacteriological Work.—During the year under report the following samples were examined bacteriologically:—

36 Samples of drinking water.
23 Samples from septic tanks.
3 Samples from Breach Candy Swimming Bath.

The bacteriological examination consisted in determining the total number of aerobic bacteria growing on agar per cubic centimetre of the sample and the smallest quantity thereof containing aerobic bacteria capable of fermenting MacConkey's bile-salt-lactose-neutral-red-broth with the production of acid and gas. These bacteria were grown at 37°C. for two days.

Drinking Water.—Fourteen samples of drinking water were derived from wells, seven from tanks, and twelve from taps. The extremes of bacteriological results shown by these samples are given in the following table :—

Source of water	Total number of bacteria per cc.	Least quantity (cc.) of samples fermenting MacConkey's broth
Well	150 to 320,000	0·000001 to 10·0
Tank	4 to 10,000	0·001 to 10·0
Tap	10 to 2,000	0·01 to 10·0

Assuming that absence of lactose-fractors in 10 cc. of a sample of water and the presence of 200 or less bacteria per cubic centimetre thereof indicate the sample to be of a fairly good quality, there were two tank water samples, three tap water samples, and one well water sample that came in this category.

Swimming bath.—The three samples of water from the Breach Candy Swimming Bath were found to be of fairly good bacterial purity; the total number of bacteria was below 50 per cubic centimetre and the quantity of sample fermenting MacConkey's broth was 1 cc. and above.

Septic Tanks.—Of the twenty-three samples from septic tanks, twenty were from the Dadar Septic Tank installation. Their examination was undertaken on behalf of the Analyst engaged by the Bombay Improvement Trust, who was experimenting to ascertain whether chlorination would remove the nuisance created by hydrogen sulphide and complained of by the neighbouring localities.

Indole.—It is interesting to note that the Cotton Wool Plug Test for Indole described in the Annual Report for the year 1920 has been recommended to be used in a routine way by the Society of American Bacteriologists in their latest Manual of Methods for Pure Culture Study of Bacteria. The Ehrlich reagent (*i.e.*, Boehme's Solution No. 1) was found to work more effectively when diluted with an equal quantity of rectified spirit. This point, which was observed by Major Malone and Dr. Gore, had escaped mention so far.

Antirabic Department.

During the year 1,332 persons applied for antirabic treatment in this Institute :—718 underwent the full course, 156 discontinued, and

458 were only given advice. The statistics of the 718 persons treated are being shown in details in the tables given in the Appendix. None died during treatment, or less than 15 days after the completion of the treatment; six died more than 15 days after the completion of the treatment. The latter, classed as "failures," give a failure rate of 0·84 per cent.

Disinfection of wounds is carried out in this Institute as a routine. Out of 1,332 persons that appeared at this Institute for antirabic treatment, 1,140 had their wounds opened up and disinfected. Of these only 690 were given antirabic treatment, the remaining 450 being advice cases. Of the 690 cases, 3 succumbed to hydrophobia in spite of early disinfection, giving a failure rate of 0·43 per cent. Of the remaining 192 that were not disinfected for certain reasons, chiefly because they came too late or no open wounds were seen on arrival, 184 were given antirabic treatment, the remaining 8 being advice cases. Among those three developed hydrophobia and died, giving a failure rate of 1·63 per cent. If we compare the failure rate between the disinfected and non-disinfected, it would seem that some reduction of mortality appears to be due to early disinfection and that this measure is worthy of further study. Details of persons who died of Hydrophobia are given in Table VII.

Out-centres.—4,254 treatments were issued to the various out-centres. Table VIII gives the statistics of persons treated at the various centres. It would not be out of place to mention that the submission of returns from the various centres has not been very satisfactory. Steps are being taken to impress on the out-centres the absolute necessity of forwarding accurate periodical returns.

Veterinary.—152 treatments were issued to various Veterinary Surgeons in the Presidency for treating animals bitten by rabid animals.

Examination of brains for rabies.—The following is the statement of the examination of brains of dogs and other animals for the diagnosis of rabies during the year 1928:—

Bombay—			
Through the Veterinary College	..	...	50
Through the Bombay Police	...	...	15
From private owners	...	...	3
From other places in Bombay	...	...	18
From outstations	..	...	80
	Total	...	166

The result of examination of these animals was as follows :—

		Percentage.
Received in a condition unfit for examination	11	6·63
No evidence of rabies	41	24·70
Negri bodies found, *i.e.*, brains obtained from rabid animals	114	68·67
Total ...	166	

Vaccine Department.

Plague—

6,259 brews of 1000 cc. each were decanted.

229,732 bottles (approximate) were filled with vaccine, labels printed for, labelled, numbered and sealed.

14,769 bottles were tested for sterility.

1,075,150 doses of the prophylactic were in all sent to the despatch department for packing.

Antirabic vaccine—

385 brews were decanted.

44,150 bottles were filled :—

55	bottles of	100 ccs.
6,870	,, ...	25 ccs.
7,670	,, ...	10 ccs.
29,555	,, ...	5 ccs.
44,150		

929 bottles were tested for sterility.

80,345 doses or 5,739 treatments were sent to the Despatch Department.

Other vaccines prepared—

4,085 ccs. mixed T.A.B. vaccine.

11,170 ccs. cholera vaccine.

Dead Emulsions prepared—

1,300 ccs. Typhoid.

300 ccs. Paratyphoid A.

300 ccs. Paratyphoid B.

Stock cultures—

271 various stock cultures were subcultured at least once a month.

13 special cultures of *pneumococci* were subcultured on special media every 4 days.

(Blood medium was prepared from time to time for the above cultures.)

Miscellaneous Work—

Decanting, 4 flasks of bile peptone glycerine,
7 „ carbolized saline,
3 „ ox bile,
1 „ normal saline,
3 „ citrate saline,
2 „ acid broth,
1 „ distilled water,
2 „ hydnocarpus oil,
5 „ horse serum,
1 „ chicken cholera,
1 „ litmus,
1 „ for immunising rats for potency,
19 „ cholera vaccine,
6 „ T. A. B.,
4 „ Dead emulsion of Typhoid,
1 „ „ „ Para A,
1 „ „ „ Para B.

Testing for Sterility—69 bottles of various vaccines, etc.

Inoculation—643 persons were inoculated against plague.

Teaching—two medical men were taught the technique of plague inoculation.

Articles supplied—vaccines :—

Kind.	No. of indents.	Quantity supplied.
Mixed T. A. B.	97	2102 ccs.
Cholera	73	9821 ccs.
Dead Emulsions :—		
Typhoid	32	244 ccs.
Para Typhoid A	28	181 ccs.
„ „ B	28	183 ccs.
„ „ C	3	6 ccs.
Horse serum	2	62 ccs.
	Times.	
Cultures :—B. acidi lactici ...	2	
V. choleræ ...	4	
B. diphtheriæ ...	2	
B. dysenteriæ Flexner ...	1	
B. dysenteriæ Shiga ...	3	

		Items.
Cultures—*contd.*	B. enteritidis Gærtner ...	1
	Micrococcus melitensis ...	1
	B. pestis ...	2
	B. paratyphosus A. ...	4
	B. ,, B. ...	4
	B. ,, C. ...	2
	B. tuberculosis ...	1
	B. typhosus ...	6

Snake venom :—3 indents were complied with for Cobra and Daboia venom.

Snakes :—30 Cobras, 4 Russell's Vipers, 4 Kraits were received during the year. On an average 16 Cobras and 14 Russell's Vipers were taken out fortnightly for extraction of venom, and feeding.

GENERAL DIAGNOSTIC DEPARTMENT.

	+	−	Total.
Blood smears for malaria	51	119	170
Blood smears for spirilla of rat bite fever ...	1	7	8
Blood smears for Microfilaria	2	5	7
Blood smears for Leucocyte count	...	...	137
Blood cultures for plague	...	...	84
Blood cultures for other organisms (Typhosus) ,etc.	...	...	11
Blood for Widal for T. A. B.	58	138	196
Blood for Widal for M. M.	...	3	3
Wassermann reactions	574	1176	1750
Sachs Georgi reactions	531	1219	1750
Kahn's reaction	128	342	470
Formol Gel reaction	23	6	29
Blood groupings	...	...	70
Autogenous vaccines	...	...	200
Sputum for T. B.	42	77	119
Sputum for other organisms	...	...	119
Scrapings for Leprosy bacilli	33	37	70
Swabs for diphtheria smears	1	15	16
Swabs for diphtheria cultures	1	15	16
Smears for Gonococci	8	19	27
Smears for B. pestis	28	39	67
Urine for general analysis	...	...	160
Urine for cultures	...	...	87
Fæces for ova	...	...	148
Fæces for cultures	...	...	112

	+	−	Total.
Cultures identified	...	...	191
Pus for cultivations	...	...	79
Cerebro spinal fluid for Meningococcus ...	...	16	16
Cerebro spinal fluid for T. B. and other organisms	...	16	16
Snakes identified	...	...	19
Snake bites treated	1	2	3
Disinfectants tested	...	...	7
Salvarsan injections given	...	...	12
Animal experiments carried out	...	...	26
Scrapings for treponema	2	7	9
Fæces for cholera, typhoid and dysentery	...	...	23
Blood smears for L. D. bodies	2	1	3
Colloidal Gold Test	...	...	1
Peritoneal fluid examined	...	...	1
Smears for spermatozoa	1	...	1
Surra in Horse's blood	3	3	6
Blood transfusion	...	...	2
Worms identified	...	...	2
Blood smears of dogs for tryps	...	4	4
Blood smears of dogs for Piroplasms		3	3

1750 bloods were examined for Wassermann and Sachs Georgi reaction. The Wassermann reaction was positive in 574, whereas Sachs Georgi was positive in 531 only or 92·5 per cent. 470 of these were also tested by Kahn's reaction, and only 95 per cent. of those that gave a positive reaction, reacted positively to Kahn's Test. The Wassermann reaction still proved to be the more sensitive of the three.

Work on bacteriophage was carried on all along the year and a strain of *B. dysenteriæ* Sonna was isolated from one of the local cases.

Seeding of flasks for plague prophylactic manufacture was done.

Pathological Section.

Pathological tumours received and examined during the year 1928 :—

Non-malignant tumours	...	46
Malignant tumours	...	87
Tissues found to be normal	...	1
Miscellaneous pathological conditions ...	...	310
Received in a state unfit for examination	...	6
Total	...	450

Table showing the classification of malignant tumours and their situation

	Carcinoma.	Epithelioma.	Sarcoma.	Endothelioma.	Rodent ulcer.	Malignant embryoma.	Total.
Abdomen	1	...	...	...	...	.....	1
Anus	...	1	...	...	...	.....	1
Arm	...	...	1	1	...	.....	2
Axillae	2	...	...	...	...	.....	2
Breast	17	...	...	...	...	.....	17
Back	...	...	1	...	...	.....	1
Bladder	1	...	...	...	...	.....	1
Cervix	4	...	...	...	...	.....	4
Cheek	...	3	...	...	...	.....	3
Chest	...	...	1	...	...	.....	1
Clavicle region ...	...	...	1	...	...	.....	
Elbow	...	...	1	...	...	.....	1
Oesophagus	2	...	...	...	...	.....	2
Eye	...	...	1	...	1	.....	2
Face	...	1	...	...	1	.....	2
Fallopian tube ...	1	...	...	...	...	.....	1
Foot	2	3	2	...	...	.....	7
Groin	1	...	...	...	...	.....	1
Jaw	...	2	...	...	...	.....	2
Leg	...	...	1	...	...	.....	1
Lip	...	2	...	...	1	.....	3
Liver	1	...	2	...	...	.....	3
Lung	...	...	1	...	...	.....	1
Neck	1	...	...	...	...	.....	1
Nose	...	1	1	...	...	.....	2
Orbit	...	...	1	...	...	.....	1
Pelvis	1	...	...	...	...	.....	1
Penis	...	1	...	...	...	.....	1
Scrotum	...	1	...	...	...	.....	1
Shoulder	...	...	1	...	...	.....	1
Sub-max region ...	...	...	1	...	...	.....	1
Supra Clavicular ...	1	...	...	...	...	.....	1
Temple	1	...	1	...	...	.....	2
Testis	...	...	...	...	...	1	1
Tongue	...	3	...	...	...	.....	3
Tonsil	...	...	1	...	...	.....	1
Thigh	...	...	3	...	...	.....	3
Uterus	4	...	1	...	...	.....	5
Vulva	...	1	...	...	...	.....	1
Total ...	40	19	22	1	3	1	86

ANTI-PLAGUE INQUIRY

I.—Observations on Cultural Characters of B. Pestis.

In the report for 1925 it has been stated that *B. pestis* was found to be one of the few Gram negative achromogenic ærobic bacilli which give a positive nitrite reaction.

Further experience confirmed this finding, and since it is a protein reaction, it was added to the carbohydrate reactions introduced by Colonel Mackie in the system of testing plague cultures used in the manufacture of Haffkine's prophylactic.

However, it was found that the object aimed at was not gained in its entirety. There were occasions when either at the second or the third examination of the seed it was found contaminated.

Since blood agar is now made easily and readily available in the form of the superimposed blood agar slopes as reported last year, this medium was routinely used in the study of cultural characters of *B. pestis*.

The very first few experiments showed that when equal quantities of successive dilutions of a broth culture of *B. pestis* were planted on a standard nutrient agar, agar made with the official broth used in the manufacture of anti-plague vaccine, and on the superimposed blood agar slopes, it was found that when blood agar would show colonies of *B. pestis* in high dilutions and in increasing numbers in the low dilutions, the standard agar and *official* agar showed a few colonies in the lowest dilution or one above that also. Further experiments showed that the plague bacillus grew better on the *official* agar than on standard agar, though the growth on both these agars was far poorer than on blood agar.

Dr. Gore was able to show that the difficulties experienced by several workers in effecting a colony count of *B. pestis* was due to these workers having used unsuitable media.

In order to establish the purity of a bacterial culture it is essential to secure numerous discrete colonies thereof on the surface of a solid medium. For this purpose ordinary media were found to be of little avail, unless they were heavily sown. In this case, discrete colonies were obtained only as a matter of chance and never routinely. On blood agar, when a series of dilutions were sown, gradual increasing numbers of colonies were obtained as a matter of course and, therefore, the superimposed blood agar slope was routinely used to secure numerous discrete colonies in each and every case submitted to examination for purity.

When thus examined a few seed cultures were found which gave a positive nitrite reaction, and correct carbohydrate reactions; however, among the discrete colonies obtained on blood agar there were colonies which looked more or less like plague, but on further incubation either remained small in size or developed a slightly yellowish tinge in the centre.

Among such colonies were Gram positive cocci and small bacilli, which eluded detection by means of the liquid cultural tests and showed themselves up only on solid media.

It will thus be seen that the minimum number of tests to which a plague culture may be submitted to be declared pure culturally are the following :—

(1) Peptone culture should give a positive nitrite reaction and a negative hydrogen sulphide, and indole reactions.

(2) When a 5 mm. loopful of a broth plague culture is diluted in 10 cc. of saline, and a 5 mm. loopful of the dilution inoculated on standard agar and 1 mm. loopful of the same inoculated on superimposed blood agar, incubation for 48 hours at 37°C. should bring out numerous discrete colonies on blood agar, and none or a few only on the standard agar. The approximate quantitative relationship between a 5 mm. loopful and 1 mm. loopful is 10 : 1.

(3) Glucose and mannite are fermented with the production of acid only, while lactose and sucrose are not fermented.

These tests have given such a control over the purity of the seed that whether it was made from the rat heart blood directly as recommended by Bannermann, or from the primary subculture on agar as used by Haffkine, it made little difference in the ultimate result.

II.—Some Quantitative Determinations in connection with the Manufacture of Haffkine's Plague Prophylactic.

1. *Optimum pH for the growth of B. pestis.*—Two series of nutrient broth tubes of pH 5·8 to 7·6 were inoculated, and the number of organisms per cc. of broth counted after 48 hours incubation. The dilution was done with pipettes and suitable dilution planted on blood agar slopes. Counts per cc. of broth pH 5·8 = 30 millions; 6·0 = 30 m.; 6·2 = 50 m.; 6·4 = 44 m.; 6·6 = 60 m.; 6·8 = 62 m.; 7·0 = 72 m.; 7·2 = 55 m.; 7·4 = 60 millions.

2. *Counts of organisations in broth at weekly intervals.*—Per cc. 48 hours growth = 57 milllons; one week, 125 millions; two weeks, 74 millions; three weeks, 42 millions; four weeks, 20 millions. Several counts were done on six weeks growth, the number of viable organisms per cc. varied between 2 and 4 millions.

3. *Immunity tests on the Prophylactic.*—As a test a series of duplicate determinations were done by Dr. Naidu by his routine method on 10 brews of plague vaccine in 1927. The results of the duplicates varied in cases by 400 per cent. and more. It was thought that this variation might be due to the variable number of organisms in the dose of test virus employed, *i.e.*, 0·003 mg. of plague-spleen. Seven test viruses made and used by Dr. Naidu were tested by Major Sokhey and Dr. La Frenais. 0·003 mg. of spleen was found to contain the following number of organisms :— 120; 5,435; 250; 15,600; 26,000; 46,000; 32,000.

The enormous variations suggested the necessity of a more constant test virus. Such a test virus was prepared and its dose varied between 490 to 1,060 organisms in the 23 immunity determinations carried out on 11 different brews of the vaccine. The duplicate results obtained were much closer, but still not close enough to justify the use of the test as a standard of measurement. Work is proceeding.

III.—Parasitology of Plague.

After an interval of more than two years the investigation of plague transmission was again taken up in August 1928 by Major Webster and Dr. Chitre.

As a preliminary, some experiments in flea breeding were carried out, and a simple means has now been devised for obtaining moderate numbers of laboratory bred rat fleas of the three species found in Bombay. Carefully identified fleas of the required species, male and female, are put in a flea-proof cage along with two English mice. Sterilised rat dung and vegetable refuse are put on the floor of the cage. The fleas live on the mice and lay their eggs on the litter, which is collected and stored in flea-proof jars after 10 days. The larvae hatch out and thrive on the litter and eventually pupate inside cocoons. In about a fortnight young fleas begin to emerge and continue to appear for a week or two. If a family is raised once a fortnight, therefore, a continuous supply of fleas is available for experimental purposes.

Four double cage transmission experiments, chiefly with wild fleas, were carried out, of which three were completely negative. In the fourth and last experiment successful transmission was obtained with both female *cheopis* and female *astia* under parallel conditions.

A series of experiments under carefully controlled conditions was then designed. In each experiment 36 fleas, 6 each male and female, *Xenopsylla cheopis*, *X. astia*, and *X. brasiliensis*, were kept in a cage with a plague infected rat. On the death of the animal, the surviving fleas were collected, identified and kept singly in labelled tubes. They were then fed individually for two or more successive days on healthy rats, and the process of feeding was observed under the entomological miscroscope. So far, fourteen of these mixed flea experiments have been satisfactory, and in these a total of 528 fleas has been employed. The successful transmissions numbered three. The fleas fed on the rats had been male *brasiliensis* in two cases, and female *cheopis* in the third instance. The successful transmissions with *brasiliensis* are interesting, as this species has not previously been proved to be a vector of plague in India. Inasmuch as *brasiliensis* forms a considerable proportion of the rat flea population in Southern India, it is an important finding.

Another method of investigation employed was designed to demonstrate whether or not, both *astia* and *cheopis*, are capable of carrying on epizootic plague under optimum conditions. Two flea-proof pits were constructed and have proved satisfactory. A dozen

rats and a hundred fleas, *cheopis* and *astia* respectively, were put in the pits, and, when it was evident that flea breeding was going on, a plague infected rat was added to each pit. The progress of the plague epizootic has been closely observed in both pits, and valuable information has been obtained. By means of material from these experiments Dr. Gore has studied the fate of the plague bacillus in the different fleas, and with his accurate cultural methods, he has obtained results differing considerably from those previously accepted as correct.

The technique of the two improved experimental methods demands considerable attention to detail, and a full account will be published elsewhere in due course.

In connection with the construction of the experimental pits, an experiment was carried out to determine the high jump of wild Bombay *Rattus rattus*.

Through arrangement with the Port Health Officer, Lieut. Colonel Houston, I.M.S., rats caught on ships in Bombay harbour are received for investigation of the rat flea population.

IV.—Anti-Plague Vaccine.

(*a*) Sixty-nine experiments (Naidu and Jung) involving the use of 2,930 rats have been carried out on 58 brews of Haffkine's Plague Prophylactic manufactured in the Institute during the year, to study the potency of the different brews of the vaccine. The results of these experiments indicate that the brews have maintained an average percentage immunity of 32·73 per cent.

(*b*) Thirteen experiments (Naidu and Jung) on the relative toxicity and immunising value of Haffkine's Prophylactic and other anti-plague vaccines prepared from agar-grown cultures, have been studied in 1,480 rats. The results indicate that Haffkine's Plague Prophylactic, in a dose of 0·5 cc., confers in rats a percentage immunity of about 35 per cent. against an infection with a large dose of highly virulent plague bacilli employed seven days subsequent to immunisation; whereas the agar-grown vaccines of Farbenindustrie, Hoechst Am Main, Germany, of Pasteur Institute, Paris, and of Lister Institute, London, confer a lower degree of protection even when employed in doses which are recommended for man, as compared with 0·5 cc. of Haffkine's Prophylactic. Lustig's Nucleo-Proteid obtained from the Serum Institute, Berne, produces a degree of immunity in rats comparable with that of the Prophylactic. The details of these experiments will be found in the paper by Dr. Naidu to be published in the Indian Journal of Medical Research.

V.—Anti-Plague Serum.

Immunisation of rabbits, sheep, and calves with cultures of plague bacilli, has been carried on throughout the year.

(*a*) The therapeutic results following the intravenous administration of immune rabbit's serum, and with anti-pestiphage, which brings about the lysis of a culture of plague bacilli within two hours, show that, when the immune rabbit's serum is administered after 24 hours of infection, the mortality is nil; whereas, when the

serum is administered at the end of 48, 72, 96 and 120 hours after infection, the mortality among the treated animals is 9, 29, 32, and 33 per cent. respectively Of the infected animals treated with antipestiphage even within an hour of infection, the mortality is 65 per cent. Of the infected animals treated with antipestiphage after 24, 48, 72, 96, and 120 hours of infection, the mortality is 50, 69, 60, 70, and 85·7 per cent. respectively. The mortality among the untreated control varied from 81 to 89 per cent.

(*b*) Experiments on the therapeutic value of immune serum obtained from calves are in progress. So far, out of the twelve rabbits infected with plague in which the treatment was commenced at the end of 48 hours of infection, the percentage mortality has been 17 per cent., as compared with the twelve infected rabbits which were treated on identical lines with the Pasteur Institute Anti-plague serum in which the percentage mortality was 36 per cent. Further immunisation of calves is being continued.

(*c*) The immunisation of sheep at weekly intervals with cultures of plague bacilli has, so far, resulted in the production of a serum which produces an agglutination with a culture of *B. pestis* in a dilution of 1 in 16. Experiments are in progress to test the therapeutic value of these sera.

VI.—The duration of immunity in Bombay rats following on the experimental infection with virulent Plague Bacilli.

Experiments were carried out to study how long the immunity conferred on Bombay rats by experimental infection with virulent plague bacilli lasts. For this purpose, the survivors of last year's experiments on the susceptibility of Bombay rats to plague, have been re-infected subcutaneously with the test dose at varying intervals, namely, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 days. The results indicate that these animals are completely immune to fresh infection at the end of 300 days from the date of the original infection. The percentage mortality among those re-infected at the end of 330 and 360 days is about 19 per cent.

INQUIRY ON MATERNAL MORTALITY IN CHILDBIRTH.

I.—Anæmia of Pregnancy.

Dr. Balfour carried on the clinical side of the investigation during the last three months of the year. A marked feature was the small number of cases accompanied by severe diarrhœa. In 1926 these formed 46 per cent. of all cases seen and in 1928 only 22 per cent. The mortality in 1926 was 39 per cent. and in 1928 was 20 per cent. of all cases seen. This was, partly at least, due to the introduction of treatment with liver diet and liver extract. Very serious difficulties met with in the treatment of this disease are the facts that women frequently delay coming until almost moribund, and that the only drug so far found successful, liver extract, is too expensive for general use. What is required is, propaganda to impress on the people the need for early treatment and also the formation of a

central fund to which well-to-do people will contribute and which will enable poor women suffering from the disease to be efficiently treated.

(*a*) *Animal Experiments.*—The results of our animal experiments may be summarised as follows :—

(1) *With Bacillus Welchii.*—The first series of experiments consisted of subcutaneous inoculations with whole living cultures of *B. welchii* or their toxins.

The second series consisted of feeding with living broth cultures of *B. welchii* with and without previous artificial ulceration of the gastro-intestinal mucous membrane by repeated doses of sodium fluoride according to the method of Khan and Torrey.

The third series consisted of intravenous inoculation with Chamberland filtrates of *B. welchii.*

Whole living cultures of *B. welchii* given subcutaneously or intraperitoneally in repeated doses produced only a slight anæmia though the injections proved fatal in nearly all the animals. The supernatant liquid obtained by centrifuging *B. welchii* broth culture and given either subcutaneously or intraperitoneally did not produce an immediate effect ; but, in some cases, when repeated doses were given a fairly well marked anæmia was produced resembling the pernicious type in man, and in some cases death supervened without anæmia. This anæmia was characterised by remissions when the injections were discontinued. That the supernatant fluid still contained living organisms was shown by the recovery of *B. welchii* in pure culture from heart blood on *post-mortem* examination.

Filtrates obtained by passing *B. welchii* broth culture through Chamberland filters when given subcutaneously or intraperitoneally produced no anæmia, and were not pathogenic to the adult animals.

B. welchii culture and Chamberland filtrates were pathogenic to the unborn young even when the animal herself remained healthy. The young were invariably still-born or died within a fortnight from birth.

Feeding with repeated doses of whole living culture of *B. welchii* even when each dose was preceded by sodium fluoride given orally also failed to produce anæmia. If at the same time the animals were kept without greens in diet death without signs of anæmia supervened. Treatment of pregnant animals with sodium fluoride alone or followed by *B. welchii* culture was toxic to the unborn young which were nearly always still-born or died shortly after birth.

To test the virulence of the *B. welchii* strain employed in these series, the organism was inoculated intravenously into a rabbit in a dose of 0·5 cc. Death took place after 18 hours and the organism was recovered in pure culture from heart blood, liver, spleen, etc. In a direct film made from the heart blood nearly all the red blood

corpuscles were hæmolysed. This experiment was repeated on another rabbit giving a smaller dose, *i.e.*, 0·1 cc. The animal died within 36 hours.

General conclusions.—(1) Inoculation of living cultures produced an anæmia of the pernicious type. (2) *B. welchii* cultures and filtrates, either injected or given orally, were toxic to the unborn young. (3) The series of feeding experiments with *B. welchii* cultures and their toxins offer no evidence that an anæmia can be produced in experimental animals by the absorption of *B. welchii* toxin through the gut wall. No evidence is therefore forthcoming that pregnancy anæmia is produced in this manner.

(2) *With Streptococci.*—

1. The injection of different strains of streptococcus isolated from the urine of anæmic cases produced no anæmia in the experimental animals.

2. Certain hæmolytic strains produced on inoculation death without anæmia; the same strains were pathogenic to the unborn young.

3. The non-hæmolytic strains on inoculation had no effect on either adult animal or young.

Conclusions.—Animal experiments with streptococci isolated from these cases give no evidence that the organism is of any etiological significance.

(*b*) *Study of 50 cases of anæmia of pregnancy and 4 non-pregnant cases with a similar blood picture.*—Cases were studied on lines of previous work in this department—in addition gastric analyses were made whenever possible and in the latter cases blood analyses as well.

Blood count and picture.—Approximately as before, but as the result of the use of more accurate method of hæmoglobin estimation (standards checked against known hæmoglobin solutions), the colour indices are distinctly lower than in previous years—64 per cent, *i.e.*, 32 out of 50 cases colour index above unity at first examination. As the red count rises the colour index rapidly decreases to below unity. These findings occur in both pregnant and non-pregnant cases. It is impossible to separate the uncomplicated cases from cases associated with malaria, hookworm, etc. on the blood picture. Certain complicated cases give the picture of a secondary anæmia (low colour index, no inversion in white count, etc.), but this is not so in all cases.

Response to liver extract.—23 pregnant anæmias, and 4 non-pregnant anæmias were treated with liver extract.

All the non-pregnant cases as judged by the blood count responded well to this treatment, showed the typical reticulocyte peak and signs of active blood regeneration (polychromatophilia, basophilic stippling, cabot rings, nucleated forms etc.). The cases were not true Addisonian anæmias (presence of free hydrochloric acid, a typical blood picture) but resemble the pregnant cases.

The blood pictures of the treated pregnant anæmias showed varying responses. Thirteen were treated antenatally, and of these 4 did not respond well, whereas the remaining did. Of the 4, one died before the extract had time to act; the second gave a typical response to liver extract, but hæmorrhaged from mucus surfaces on 5th day and died; the third had in addition malignant malaria and hookworm (other cases similarly complicated responded to treatment); and the fourth simply did not respond. With two exceptions the post natal cases gave typical good pictures of blood regeneration. The exceptions were firstly a case of hookworm infection, and secondly a very severe case that was treated for 21 days but never showed any sign of blood regeneration, this latter case was possibly septic. The complicated cases that failed to respond had a microcytic rather than a macrocytic type of anæmia (actual measurements of cells made).

Blood Analyses.—Not sufficient analyses have been done for any deductions to be made, but certain findings may be noted. The figures in no way suggest absorption from the gut; although the nitrogen values tend to be on the high side, the chloride values are also high. If the cases are divided according to the degree of anæmia it is found that the nitrogen values are higher the greater the anæmia, the chlorides also as would be expected from the greater percentage of plasma, whereas the calcium values increase as the anæmia decreases. Using a small series of osteo-malacia cases as a control, the nitrogen and chloride values suggest that in the anæmic cases, even when the counts approximate to those of the latter series, the non-protein nitrogen and chloride values are raised. Quite a number of the cases have low inorganic phosphate and calcium values, the significance of which is being correlated with certain dietetic findings.

Urinary Analyses.—The urine was examined chemically in all cases and with very few exceptions showed no abnormality.

Test meals.—The question of achlorhydria has been worked out. The presence of free acid in nearly all the cases divides these from true Addisonian anæmia. The actual findings showed 11 antenatal uncomplicated cases—all had free acid present during the meal; seven antenatal complicated cases; two no free hydrochloric acid; of these one with high chloride was a case of malaria and syphilis —this case had high chlorides, and the second was complicated by malaria and hookworm and had low chlorides (32 per cent.). Five post natal cases—one only no free acid but 40 per cent. chlorides; of the three non-pregnant cases two had no free acid at the first meal but it was present when the meal was repeated. Some of the pregnant cases were tested when the counts were 1,000,000 and the patients still very ill, so that the findings would suggest that achlorhydria is uncommon, even during the acute stages of the disease.

Bacteriological and other findings.—The urine, resting gastric juice and sometimes swabs from the gums were cultured whenever possible, with particular reference to the presence of streptococci and the anaerobes.

As far as the findings go there is no evidence that these organisms play a causal part in the disease.

Urine.—In 39 cases the urine was cultured, 30 of these cases were antenatal, and of these one only grew a streptococcus and this was non-haemolytic. Of 9 post natal cases cultured 5 grew streptococci, of which one strain was haemolytic. It should be noted that the incidence in the post natal cases is much higher. In the series quoted in last year's report the same incidence is seen: of 8 antenatal cases 2 grew streptococci — of 7 post natal all grew streptococci. It should be noted that 5 of the post natal and one of the antenatal occurred in the same hospital—in fact every case there that was cultured gave a growth of streptococci.

In no case was an anærobe grown from the urine.

Resting Gastric Juice.—None in the series gave a growth of either streptococci or anærobes.

Teeth.—Several cases grew streptococci from swabs from the gums.

Complications.—The incidence of malaria, hookworm and specific disease was approximately as before. With a view to determining more accurately the incidence of malaria films have been made from the placental blood, but an insufficient number of cases have been studied as yet to report upon.

As no evidence has been obtained that the disease is due to any one infective agent, though certain cases would appear to be secondary to malaria and hookworm, a dietetic study has been begun and the evidence obtained is such that it has been thought worth while putting up some animal feeding experiments; these are now being carried out.

II.—Osteomalacia.

Investigations into the blood changes in Osteomalacia are being carried out, the findings being correlated with the result of treatment with cod liver oil or ultra violet light. Up to date the results differ from those reported from China in that the cases under consideration have, with one exception, shown low inorganic phosphate values with only slightly reduced serum calcium values. A case admitted with tetany gave markedly lowered serum calcium values. Improvement was obtained under both forms of treatment with corresponding changes in the blood chemistry. This investigation is being continued.

III.—Maternity Condition of Women Mill Workers.

This investigation was somewhat interrupted by the prolonged strike of nearly seven months, but the numbers under observation were brought up to 428 by the end of the year.

IV.—Age of Primiparæ in India.

Further observations were made. The number of primiparæ watched in Bombay Hospitals was brought up to 1,112. The average age was 20·5 years, 12 per cent. were below 16 years of age and the youngest was 14 years of age.

V.—Early infant mortality

An enquiry had been begun with the object of throwing light on the early infant mortality in Bombay. The mothers of all children born in the Cama Hospital with a birth weight below 5½ lbs. were put under observation, and a nurse was engaged to watch the further progress of the infants. Unfortunately her salary had to be discontinued from the end of the financial year owing to the need for retrenchment.

VI.—Diets

Some information has been collected with regard to the diets of patients suffering from anæmia of pregnancy. This is being continued.

TUBERCULOSIS INQUIRY

Surgical Tuberculosis in India.—Investigation of the nature of organisms causing the so-called surgical type of tuberculosis in human beings in India was continued. A large proportion of cases of this type of disease in European countries where cattle tuberculosis is prevalent is known to be caused by the bovine bacillus. Previous study by Dr. Soparkar of 65 indigenous cases comprising 40 of cervical adenitis, 8 of axillary gland tuberculosis (20 out of which were from children under 16 years) and 17 of pulmonary disease, showed that the human tubercle bacillus was responsible for the disease in all cases. One of the strains recently isolated from tuberculous cervical glands in a girl from the Cama Hospital, Bombay, however, was found to be of the bovine type. This is the first instance to be recorded in which bovine tubercle bacillus is found to cause human tuberculosis in India, and is of interest in connection with another recent finding of the comparatively high incidence of tuberculosis found among indigenous cattle in some parts of India reported previously. Material from 32 more cases of glandular and bone and joint tuberculosis and pulmonary disease has been collected and the work is in progress.

Filtrability of the Tubercle Bacillus.—Several continental workers claim to have found the existence of a filter passing form of tubercle bacillus and work on this question was commenced at Kasauli. The material to be investigated consisted of different tissues of experimental animals dying of severe tuberculosis, of cultures of varying ages, and pathological tuberculous material, *e.g.*, pus, sputum, etc., from human sources. The experiment was controlled each time by previous mixture of the material to be filtered with *B. prodigiosus*, and Chamberland L_3 candle was employed for filtration and the filtrate was subsequently used for animal inoculation and for culture. Up to now 29 experiments have been performed with material from different sources. Results of the first eight experiments performed during the early part of the year have shown no evidence of tuberculosis either in the culture media or in the animals inoculated with the filtrate except in one instance. This guineapig, which was inoculated subcutaneously

with 7 cc. of the filtrate of an emulsion of the lung tissue of a rabbit that had died of generalised tuberculosis after inoculation with a virulent bovine strain showed, when sacrificed 102 days after inoculation, tuberculous lesion at the seat of inoculation and in the neighbouring glands and spleen, and tubercle bacilli were demonstrated in the tissues. Another guineapig inoculated from the first animal died in 94 days of generalised tuberculosis, and a strain has been isolated and is under study. One of the culture tubes inoculated with the filtrate used in this experiment showed a minute colony of contaminating organisms ten weeks after inoculation. Whether the contamination was the result of defective candle or appeared subsequently it is difficult to say but the other tubes inoculated with the same filtrate remained sterile. This is the only instance in which the animal inoculated with the filtrate showed a tuberculous lesion. The other experiments have been done recently and sufficient time has not yet elapsed to judge of the results.

Experiments with B. C. G.—Experiments were made with B. C. G. to test its (I) non-pathogenicity, (II) immunising property. The strain was obtained from Professor Calmette in 1927.

Experiments on Guineapigs (I).—Nineteen guineapigs were inoculated subcutaneously, with doses varying from 10 to 50 mgms. of a culture of B. C. G. on glycerinated potato. All the animals died in 5 to 39 days, the majority within 23 to 31 days after inoculation. Many of the animals showed considerable emaciation, loss in weight varying from 120 to 310 gms. At autopsy a specific local lesion was observed in all animals and in several of them definite tuberculous changes were produced in addition in the neighbouring glands. In no case, however, did reinoculation of the material into other animals result in lesions of greater severity. In fact after the third passage no evidence of tuberculosis could be detected in the inoculated animals, indicating that although tuberculous lesions are produced in some animals by inoculation of live B. C. G. culture, the lesions are not reinoculable and that further passage through animals does not result in increase of its virulence.

Experiments on Rabbits (I).—Thirty rabbits were inoculated with B. C. G., 12 with 50 mgms. subcutaneously and 18 with 20 mgms. intravenously. Of these four inoculated intravenously died in 12 to 20 days with definite tuberculous nodules in the lungs which on histological examination showed typical structure of anatomical tubercle. Three died in 29, 30 and 155 days of intercurrent disease and three others after 193, 195 and 264 days but on autopsy showed no evidence of tuberculosis whatsoever. One died with doubtful result which is under further investigation and the rest of the animals (19) are alive. The result shows that specific lesions are observed in the lungs of rabbits after intravenous inoculation with B. C. G. when death takes place within a few days after inoculation but they are retrogressive and tend to disappear after some time.

Immunising property of B. C. G. (II).—Six rabbits were vaccinated with 15 mgms. of B. C. G. intravenously. After two months the

surviving five were given a test dose intravenously of 1/100 (4) and 1/10 mgm. (1) of a virulent bovine culture along with controls. The two control rabbits inoculated with 1/100 mgm. died in 60 and 93 days of severe generalised tuberculosis and the third inoculated with 1/10, mgm. in 25 days. The vaccinated animals tested with the bovine virus also died of generalised tuberculosis but in their case life was prolonged, *i.e.*, for 93, 113, 183 and 187 days.

Another batch of 15 vaccinated rabbits has been tested with a virulent bovine culture but in still smaller doses, *i.e.*, 1/100, 1/1000, and 1/10000 mgm. Sufficient time has not, however, elapsed to judge of the results, although three out of 6 control rabbits have died of generalised tuberculosis within a month.

Experiments on Cattle (II).—Reference was made in the report for the previous year regarding the immunising effect of vaccination with B. C. G. on previously infected animals. Further work in this connection was done on healthy animals. A number of persumably healthy cattle were tested by the thermal tuberculin test and ten of those that had given a negative reaction were vaccinated with 100 mgms. each of a 40 days culture of B. C. G. No ill effects of the inoculation were noticed and the animals remained in good condition. One of these animals died some weeks later of other causes. The remaining 9 animals were given a test dose intravenously of 5 mgms. of a virulent strain of bovine bacilli (previously obtained from Dr. Griffith) four and a half months after vaccination. Eight out of 9 animals died of acute miliary tuberculosis in 15 to 48 days. One animal, however, remained in good condition and when destroyed 161 days after the test inoculation showed no evidence of tuberculosis. Control experiments done previously on healthy non-vaccinated animals that were inoculated intravenously with 5 mgms. of the same strain resulted in their death from acute miliary tuberculosis in 13 to 19 days. These results differ from those obtained by the Ukraine Commission.

In the experiments carried out by the Ukraine Commission reported by Professor Tzekhnowitzer intravenous test inoculation of previously vaccinated animals with mgms. of a virulent bovine culture (Vellee) failed to produce any serious disease. Nine out of 13 animals that were sacrificed at intervals of 3 to 12 months after the test dose showed on examination only a few scattered retrogressive lesions and clinically the animals were found to be in very good condition and had shown a regular increase in weight.

It may, therefore, be inferred that under conditions of the experiment very little resistance would appear to have been produced in Indian hill bulls by vaccination (subcutaneous) with 100 mgms. of a 40 days culture of B. C. G. against a test dose of 5 mgms. of a bovine strain given intravenously.

Transformation experiments.—Experiments upon transformation of tubercle bacilli of one type into another were continued by Dr. Soparkar. This subject has been engaging his attention since his first finding of a change in the character observed in the strains isolated after more than a year from several bovines that were

originally inoculated with a culture of avian tubercle bacilli three years ago. Three animals (two buffaloes and one bull) previously inoculated first with a culture of avian tubercle bacilli subcutaneously on 2nd April 1925 and later with the same strain intravenously on 10th May 1926 and the buffaloes again a year later were destroyed on 22nd Feburary 1928, 10th March 1928 and 16th August 1928, respectively, and cultures were isolated from them. Study of the cultures isolated from the buffaloes showed them to be of the original avian type but that isolated from the bull gave characters of a different, *i.e.*, of the bovine type. The difference may be accounted for, if transformation is believed to be possible, by the fact that the last inoculation in this animal was done more than two years previously while similar was not the case with the buffaloes and the strain had perhaps sufficiently prolonged residence in the body of the bull to show a change in character. Other possible explanations for the change noted have also to be considered and no definite deduction can be drawn that the result indicates a true transformation of type and further work on this important question is contemplated.

List of Papers published during the Year

1. Yeasts and Sprue; by Mackie, F. P., and Chitre, G. D., The Indian Medical Research Memoirs, Memoir No. 11, August 1928.

2. Animal Experiments and Sprue; by Mackie, F. P., and Chitre, G. D., *Ind. Jour. Med. Res.*, Vol. XVI, No. 1, July 1928.

3. The Association of Bowel Disease with Vitamin C Deficiency, by Mackie, F. P., and Chitre, G. D., *ibid.*

4. The Morbid Anatomy; by Mackie, F. P., and Fairley, N. H., *Ind. Jour. Med. Res.*, Vol. XVI, No. 3, January 1929.

5. The Microscopical changes occurring in organs after Death; by Mackie, F. P., *ibid.*

6. Liver Function in Sprue; by Sokhey, S. S., Gokhale, S. K., Malandkar, M. A., and Billimoria, H. S.; *Ind. Jour. Med. Res.*, Vol. XV, No. 3, January 1928.

7. Pancreatic Function in Sprue; by Sokhey, S. S., and Malandkar, M. A., *Ind. Jour. Med. Res.*, Vol. XV, No. 4, April 1928.

8. The Bacteriology of Sprue; by Mackie, F. P., Gore, S. N., and Wadia, J. H., *Ind. Jour. Med. Res.*, Vol. XVI, No. 1, July 1928.

9. A note on an unrecognised Bacillus isolated from Sprue Cases; by Mackie, F. P., and Gore, S. N., *Ind. Jour. Med. Res.*, Vol. XVI, No. 2, October 1928.

10. Anæmia in Sprue; by Fairley, N. H., and Billimoria, H. S., *Ind. Jour. Med. Res.*, Vol. XVI, No. 3, January 1929.

The following papers were read before the last session of the Indian Science Congress held at Madras :—

1. Animal Experiments with *Bacillus Welchii* isolated from Fæces of cases of Anæmia of Pregnancy; by Maneck M. Mehta.

2. The Superimposed Blood Agar Slopes ; by Gore, S. N.

3. The Isolation, Examination and Identification of *B. pestis* by Cultural Methods ; by Gore, S. N.

4. A case of Bovine Tubercle Bacillus Infection in Man in India; by Soparkar, M. B.

5. Tuberculosis among Swine in India ; by Soparkar, M. B.

6. Preparation of a Potent Anti-Plague Serum in India ; by Naidu, B. P. B., and Jung, S. S.

7. The Relative Toxicity and Immunising Value of Haffkine's Plague Prophylactic and other Anti-Plague Vaccines ; by Naidu, B. P. B., and Jung, S. S.

APPENDIX

TABLE I

Statistics for persons treated at Haffkine Institute, Parel, Bombay, 1922-1928

Year	Total treated	Failures		Deaths		Indians			Europeans			Cases extracted, advice cases and incomplete treatment
		Total No.	Per cent.	Total No.	Per cent.	Total No.	Deaths	Per cent.	Total No.	Deaths	Per cent.	
1922	429	4	0·93	4	0·93	365	4	1·09	64	...	...	213
1923	845	9	1·06	10	1·18	730	10	1·37	115	...	...	523
1924	587	7	1·19	10	1·70	525	9	1·71	62	1	1·61	513
1925	625	5	0·8	6	0·96	583	6	1·02	42	...	...	355
1926	648	4	0·6	5	0·76	588	5	0·87	60	...	...	611
1927	729	5	0·68	6	0·82	678	6	0·88	51	...	...	644
1928	718	6	0·83	6	0·83	679	6	0·88	39	...	...	614

TABLE II

Classification of patients by probability of rabies in the biting animals

Class	Sub-class I Bitten on head or face	Sub-class II Bitten through bare skin anywhere except on the head or face	Sub-class III Bitten through clothing	Sub-class IV "Licks" on any part of the body	Total	Deaths	Mortality per cent.
Class A—Bitten or licked by animals proved rabid ...	2	34 (2)	3	30	69	2	2·89
Class B—Bitten or licked by animals certified rabid ...	1	17	5	1	24	...	...
Class C—Bitten or licked by animals suspected rabid ...	9	480 (4)	131	5	625	4	0·64
Total ...	12	531	139	36	718	6	0·83
Europeans							
Class A—Bitten or licked by animals proved rabid ...	1	5	...	14	20	...	...
Class B—Bitten or licked by animals certified rabid ...	...	...	...	1	1	...	...
Class C—Bitten or licked by animals suspected rabid ...	...	10	4	4	18	...	...
Total ..	1	15	4	19	39	...	...
Indians							
Class A—Bitten or licked by animals proved rabid ...	1	29 (2)	3	16	49	2	4·08
Class B—Bitten or licked by animals certified rabid ...	1	17	5	...	23	...	...
Class C—Bitten or licked by animals suspected rabid ...	9	470 (4)	127	1	607	4	0·65
Total ...	11	516	135	17	679	6	0·88

TABLE III

Cases developing hydrophobia after 15 days of completion of treatment "Failures"

Case No.	Sex	Age	Animal	Number and position of the bite	Whether on bare skin or through clothing.	Whether cauterized or not	Date of commencing treatment	Number of days late	Duration of illness	Interval between bite and death
206	M	30	Dog ...	3; leg	Bare...	Uncauterized.	22nd February 1928.	5 days	2 days	35 days
851	M	12	,, ...	1; arm	,, ...	Cauterized ...	3rd Aug. 1928...	1 day	1 day	78 days
1098	M	11	,, ...	3; leg	,, ...	Do. ...	27th October 1928.	0 day	1 day	70 days
1101	M	7	,, ...	3; leg	,, ...	Do. ...	27th October 1928.	0 day	2 days	77 days
1134	M	22	,, ...	4; arm	,, ...	Do. ...	6th November 1928.	16 days	2 days	74 days
1206	M	14	,, ...	4; arm	,, ...	Do. ...	25th November 1928.	15 days	1 day	47 days

TABLE IV

Showing the animals which bit, scratched or licked the patients

Species of animal	Number of persons bitten or scratched or licked	Deaths
Dog	694	6
Jackal	3	.....
Monkey	2	.....
Cat	12	.....
Pig	1	.....
Human beings	6	.....
Total ...	718	6

TABLE V

Showing the number of persons who came for treatment each month during the year 1928

Month	Discontinued treatment	Europeans		Indians	
		Treated cases	Advice cases	Treated cases	Advice cases
January	18	5	3	65	24
February	13	3	5	66	40
March	20	5	2	63	49
April	15	2	3	62	32
May	16	7	5	66	40
June	12	3	2	54	35
July	9	2	4	46	40
August	11	1	2	44	30
September	7	3	...	59	33
October	8	1	3	46	34
November	13	3	4	58	25
December	14	4	7	50	36
Total ...	156	39	40	679	418

TABLE VI

Total number of patients treated at Parel according to provinces from which they came

Province	Europeans	Indians	Total
Bombay City	38	622	660
Bombay Presidency	1	51	52
Central Provinces	.....	1	1
Indian States	.....	1	1
Goa	.....	4	4
Total ...	39	679	718

TABLE VII

Details of persons who died of Hydrophobia.

Case I.—S. V. P. was bitten by a dog at Alibag, Bombay Presidency, on 17th February 1928. Two other persons were also bitten by the same dog. Arrived here for treatment *five days later*. No open wounds were seen on admission. Three scars were seen on the right leg. The scars were not opened up. Underwent a full course of anti-rabic treatment. Died of Hydrophobia on 21st March 1928.

Case II.—D. and D. was bitten by a stray dog on 10th November 1928. Arrived here for treatment on 25th November 1928, *15 days late*. He had four deep septic wounds with severe œdema involving the whole arm. He was sent to the K. E. M. Hospital for admission and treatment. The wounds were not disinfected here. Underwent the full course of treatment and developed hydrophobia on 26th December 1928.

Case III.—A. P. D. bitten by a suspected rabid dog on 20th October 1928 at Goa. Reported here for treatment on 6th November 1928 (16 days late). Four deep septic wounds on the right wrist. The wounds were opened. Given the full course of treatment. Developed hydrophobia and died on the 3rd January 1929.

Case IV.—N. M. was bitten by a dog proved rabid on 27th October 1928. Reported here for treatment five hours later. Three deep wounds on left leg. Wounds were opened and disinfected. Underwent the full course of anti-rabic treatment. Developed hydrophobia on 10th January 1929.

Case V.—C. D. aged 12 was bitten by a dog at Bandra on 2nd August 1928. From history, the dog was undoubtedly rabid, as it had bitten two others a few hours later. The dog was killed. Appeared for treatment on 3rd August 1928, 24 hours later. One deep lacerated wound involving the deep tissues situated near the left elbow. The wound was incised and disinfected. Developed Hydrophobia on 16th October 1928.

Case VI.—K. M. bitten by a dog on 27th October 1928. This dog had bitten several people, and was killed by the police. The person was taken to the J. J. Hospital immediately and the wound 4" × 2" × ½" over the ankle was stitched. On arrival here (two hours later) the sutures were removed and the wound disinfected. Full course of treatment was given. Developed hydrophobia on 4th January 1929.

TABLE VIII

Statement of cases treated at different centres during 1928

Name of place	Number of persons for whom vaccine was issued	Number of persons who received the full course of treatment	Number of persons not fully treated	Number of reports received from persons alive and well for at least three months from date of treatment	Died during treatment	Died less than fifteen days after completing treatment	Died more than fifteen days after completing treatment	Number of persons from whom no reports received
Ahmedabad ...	665	604	61	...	1	...	...	603
Ahmednagar ...	125	125	...	...	...	...	...	125
Ajmere ..	128	123	3	95	1	...	...	27
Akola ...	159	151	8	130	...	1	...	20
Baroda ...	96	78	18	26	...	...	1	51
Bombay (Byculla and Colaba) ...	364	363	1	153	...	...	...	210
Belgaum ...	156	148	8	113	...	1	...	34
Cambay ...	1	1	...	1	...	...	...	...
Chhota Udaipur .	8	8	...	8	...	...	...	...
Dharwar ...	5	5	...	5	...	...	...	...
Hoshangabad ...	251	232	19	37	...	...	1	194
Hubli ...	27	27	...	27	...	...	...	...
Hyderabad ..	36	35	1	8	...	...	...	27
Indore ...	369	397	22	54	...	...	1	342
Jalgaon ...	51	45	6	21	...	1	...	23
Jhansi ...	68	60	8	26	...	...	...	34
Jubbulpore ...	334	324	10	30	1	2	2	289
Karachi ...	120	116	25	112	...	...	...	4
Kolhapur ...	21	21	...	21	...	...	...	...
Karwar ...	4	4	...	3	...	...	1	...
Mhow ...	14	14	...	14	...	...	...	...
Nagpur ...	303	271	32	101	2	...	...	168
Nasik ...	95	91	4	72	...	...	1	18
Nasirabad ...	19	19	...	19	...	...	...	...
Nova Goa ...	73	70	3	66	1	...	2	1
Poona ...	503	366	137	21	...	...	2	343
Raipur ...	74	67	7	...	...	...	...	67
Rajkote ...	456	424	32	256	2	6	...	160
Ratnagiri ...	9	9	...	9	...	...	...	...
Quetta ..	44	33	4	16	...	...	...	17
Sholapur ...	24	15	9	..	...	...	...	15
Saugor ...	4	4	...	4	...	...	...	...
Surat ...	181	192	49	55	...	...	4	73
Total ...	4,787	4,382	467	1,503	8	11	15	2,845

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